REMARKS

Claims 15, 16, 18, 20 - 51 and 58 - 79 are pending in the present application. Claims 15, 18, 24, 27 - 29, 36, 47 - 51 and 58 have been amended and claims 1 - 14, 17, 19 and 52 - 57 have been canceled. A marked-up version of the amended claims is provided as an appendix hereto. Claims 63 - 79 are added by this amendment, and new claims 63, 73 and 76 are independent claims. Applicants reserve the right to file further continuation and divisional applications on any subject matter disclosed in the application but not presently claimed.

Claim 15 has been amended to incorporate the limitation of now canceled claim 17 and to define the phrase "carbon source" as disclosed at page 7, lines 18 - 27 of the specification. Claim 18 has been rewritten in independent form and incorporates the limitation of now canceled claim 19. Correction of minor grammatical mistakes have been made in Claims 24 and 36. Additionally Claims 24 and 27 - 28 now refer to an enzyme or an enzyme having a specific activity as opposed to the enzyme as an activity itself. Claims 47 - 51 and 58 have been amended to correct claim dependency.

New independent claim 63 is directed to a process for the non-fermentative production of 2-KLG including three enzymatic oxidation steps and one reductase step wherein glucose as a carbon source is oxidized to gluconate by GD in the first oxidation step. In this embodiment an oxidized co-factor, required by the GH and a reduced form of the co-factor, required by the reductase are recycled between the glucose oxidizing step and the reducing step. Claims 64 - 72 are dependent on claim 63 and support for the claims may be found in the original claims.

New independent claim 73 is also directed to a process for the non-fermentative production of 2-KLG including three enzymatic oxidative steps and one reductase step wherein glucose as a carbon source is oxidized to gluconate by GD in the first oxidation step and an oxidized co-factor required by GH and a reduced form of the co-factor, required by the reductase are recycled between the glucose oxidizing step and the reducing step. However, the process proceeds in an environment comprising exogenously added 2,5-DKG reductase. Support is found at least at page 11, lines 7 - 9. Claims 74 and 75 are dependent on claim 73 and support for the claims may be found in the original claims.

New independent claim 76 is directed to a process for the non-fermentative production of 2-KLG in an environment comprising host cells, including enzymatically oxidizing a carbon source selected from the group consisting of glucose, gluconate, and 2-keto-D-gluconate by at least one oxidative enzymatic activity to an oxidation product wherein said oxidative enzymatic activity requires an oxidized form of an enzymatic co-factor; and enzymatically reducing said

oxidation product by at least one reducing enzymatic activity to 2-KLG wherein said reducing enzymatic activity requires a reduced form of said enzymatic co-factor wherein the oxidized form of said co-factor and the reduced form of said co-factor are recycled. Support for claim 76 is found in original claims 15 and 17 and page 7. Claims 77 -79 are dependent on claim 76 and support for these claims is found in the original claims.

Applicants have amended the specification at page 21, Example II, wherein D-gluconate dehydrogenase was inadvertently referred to as opposed to glucose dehydrogenase, and therefore the specification should not longer be objectionable.

Additionally the informality of claim 38 has been corrected.

Claims 24 and 27 have been rejected under 35 U.S.C. §112, second paragraph. Originally claim 24 recited an enzyme which is "a dehydrogenase activity" and original claim 27 recited an enzyme which is "a GADH activity". The phraseology of the claims has been corrected to refer to an enzyme which has a specific activity as opposed to the enzyme as an activity itself. Withdrawal of this rejection is kindly requested.

Claims 15 - 46, 59 - 62 and 47 - 51, 58 (in part) have been rejected under 35 U.S.C. §112, first paragraph.

The Examiner states,

> "the specification fails to describe representative reactions producing KLG, other than the reaction of glucose oxidation to gluconate; gluconate oxidation to KDG; KDG oxidation to DKG; and DKG reduction to KLG and therefore a starting material defined as a "carbon source" is not supported by the specification."

Independent claims 15 and 18 have been amended to recite the definition of a "carbon source" as found at page 7, lines 18 - 27 of the disclosure. As understood by those of ordinary skill in the art, acidic derivatives of saccharides may exist in a variety of ionization states depending on the surrounding media. Applicants state that when the term carbon source is referred to in the claims, it is intended to include the broad meaning of the term. As taught in the specification the use of such a term is intended to include all ionization states. As a nonlimiting example, gluconic acid and gluconate refer to the same organic moiety and further it is known that gluconic acid can exist not only in the unionized form but also in an ionized form, such as a sodium, potassium or other salt.

Applicants assert the rejections under 35 U.S.C. §112, first and second paragraphs should be withdrawn.

Claims 15 - 51 and 58 - 62 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Light et al.

The Examiner states,

> Light et al. (US Patent 4,758,514) teach the pathway glucose-2-KLG (column 1, lines 16 - 29). They further teach the production of 2-KLG from glucose by Erwinia cell transformed with 2,5-DKG reductase gene (column 17, line 62 through column 20, line 5, Examples 5 and 6). This process comprises enzymatical oxidation of glucose by Erwinia into DKG and enzymatical reduction of DKG to 2-KLG. Since enzyme involved in oxidation of glucose to DKG are known in the art it would have been obvious to the one of ordinary skill in the art at the time the invention was made to carry out non-fermentative oxidation of glucose into DKG using purified enzymes or cells transformed with a DNA encoding an enzyme. One would have been motivated to use non-fermentative oxidation of glucose into DKG as a matter of convenience."

Light et al. disclose that the enzymatic conversion of glucose to DKG can be carried out in a single organism including organisms of the genus Gluconobacter, Acetobacter or Erwinia. Prior to the Light et al. invention the further conversion of DKG to KLG would utilize at least another organism or killed culture thereof and would be limited to batch-wise processes. The Light et al invention includes the production of substantially pure DKG reductase, and in one aspect the construction of an expression vector including the DKG reductase from Corynebacterium wherein the gene encoding the reductase is cloned into a host capable of converting glucose or other available metabolite to DKG such hosts include Erwinia.

Clearly Light does not disclose a process for production of 2-KLG wherein at least one oxidative enzyme activity requires an oxidized form of a co-factor and the reducing enzymatic activity requires a reduced form of the co-factor wherein the reduced and oxidized co-factors are recycled between the oxidizing step and the reducing step. At column 7, Light et al. discloses that the DKG reductase requires NADPH and that sources of electrons for the reduction of the coenzyme may be provided by any reduced substrate in contact with an enzyme for its oxidation. Furhter Light et al. disclose that other systems for regenerating NADPH cofactors are known in the art using, for example H_2 as a source of reducing equivalents and lipoamide dehydrogenase and hydrogenase or ferredoxin reductase and hydrogenase as catalysts. But there is no teaching that the process for the production of KLG from a carbon source as disclosed in the instant specification could include a recycling of the co-factor between the oxidizing step and the reducing step of the glucose to KLG conversion. The advantage of Applicants' process is that enzymatic regeneration of the co-factor dependent

reductase is not at the expense of another substrate that is oxidized. Also as stated at page 11, lines 4 - 8, this embodiment provides a means for co-factor regeneration, thereby eliminating the cost of continuously adding exogenous co-factor to the bioreactor for the production of KLG in Pantoea cells.

Claims 15 - 51 and 58 -62 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Powers et al. The Examiner states,

> "Powers (US Patent 5,795,761) teach the pathway glucose-2-KLG. They further teach that a number of microorganisms such as Erwinia, Acetobacter and Gluconobacter can produce 2,5-DKG from glucose and the second group can reduce 2,5-DKG to 2-KLG (column 1. line 25 - 61). They teach reductase A:F22Y/A272G mutant (figure 10, for example) catalyzing conversion of 2,5-DKG to 2-KLG. Since enzyme involved in oxidation of glucose to DKG are known in the art it would have been obvious too one of ordinary skill in the art at the time the invention was made to carry out non-fermentative oxidation of glucose into DKG using purified enzyme or cells transformed with a DNA encoding an enzyme. One would have been motivated to use non-fermentative oxidation of glucose into DKG as a matter of convenience."

Powers et al. is concerned with the production of DKG mutants with improved properties. One mutant characterized by Powers et al., is the double mutant F22Y/A272G which is also used by the Applicants in the present application. However, Powers et al. suffers from the same deficiency as Light et al. There is no teaching in the reference concerning a process for the production of KLG wherein the oxidized form and the reduced form of the required co-factor for the reducing step and at least one oxidization step are recycled between said steps.

It is a well known tenet of patent law that for a reference or combination of references to render an invention unpatentable, the reference or combination of references must suggest to one of ordinary skill in the art the claimed invention. Beyond looking to the reference to determine if it suggests doing what the inventor has done, one must also consider if the reference provides the required expectation of succeeding. Applicants assert the cited references offer no suggestion or motivation to provide a process for the production of KLG wherein the process includes the recycling of co-factor between at least one oxidizing step and the reducing step of said process. Applicants contend neither Powers et al. nor Light et al., render the claimed invention unpatentable and request withdrawal of all rejections under 35 U.S.C. §103(a).

In view of the foregoing, Applicants believe all claims now pending in this application are in condition for allowance. The issuance of a formal Notice of Allowance is respectfully requested. If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at (650) 846-7620.

Respectfully submitted,

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Enc: Marked-Up Version of the Amended Specification
Marked-up Version of the Amended Claims

MARKED-UP VERSION OF THE SPECIFICATION - EXAMPLE II

- - **Example II**

Example II describes the method for producing a host cell having a mutation in the naturally occurring 2-keto-D-gluconate dehydrogenase (E3).

2-Keto-D-gluconate dehydrogenase (EC1.1.99.4) from *Gluconobacter melanogenus* is purified according to the procedure of McIntire et al., (McIntire, W., Singer, T.P., Ameyama, M., Adachi, O., Matsushita, K., and Shinagawa, E., Biochem J. (1985) 231, 651 - 654) and references therein. The purified protein is digested with trypsin and chymotrypsin or other proteases to produce peptide fragments which are separated by HPLC or other techniques. Individual peptides are collected and sequenced. From the sequence, DNA probes are synthesized which will anneal to the corresponding sequence in the host organism or a related organism's genome. Using standard PCR techniques, larger fragments of the desired gene are amplified, purified and sequence. These fragments are used to hybridize to the gene and allow for cloning and sequencing of the entire gene. Once the sequence is known, the gene is deleted as described for **[D-gluconate dehydrogenase]** **glucose dehydrogenase** (GDH) in Example1.

Other methods to reduce or eliminate 2-keto-D-gluconate dehydrogenase include inhibitors (organic acids such as citrate and succinate are reported to inhibit 2-keto-gluconate dehydrogenase; Shinagawa, E. and Ameyama, M. Methods in Enzymology (1982) 89, 194-198) and changes in pH or temperature.

The enzyme activity can be assayed for activity or loss of activity using the assays described in Shinagawa and Ameyama. - -

MARKED-UP VERSION OF THE AMENDED CLAIMS

Claims 1 - 14 - canceled

15.(Amended) A process for the non-fermentative production of 2-KLG from a carbon source, comprising the following steps in any order,

a. enzymatically oxidizing the carbon source by at least one oxidative enzymatic activity to an oxidation product wherein said oxidative enzymatic activity requires an oxidized form of an enzymatic co-factor; and

b. enzymatically reducing said oxidation product by at least one reducing enzymatic activity to 2-KLG wherein said reducing enzymatic activity requires a reduced form of said enzymatic co-factor

wherein the oxidized form of said co-factor and the reduced form of said co-factor are recycled between at least one oxidizing step and at least one reducing step and said carbon source is selected from the group consisting of 6-carbon sugars, mixtures of 6-carbon sugars, 6-carbon sugar acids, and enzymatic derivatives thereof wherein said carbon source is capable of being converted to an ASA intermediate .

Claim 17 - canceled

18.(Amended) A process for the non-fermentative production of 2-KLG from a carbon source, comprising the following steps in any order:

a. enzymatically oxidizing the carbon source by a first oxidative enzymatic activity to a first oxidation product;

b. enzymatically oxidizing the first oxidation product by a second oxidative enzymatic activity to a second oxidation product;

c. enzymatically oxidizing the second oxidation product by a third oxidative enzymatic activity to a third oxidation product; and

d. enzymatically reducing the third oxidation product by a reducing enzymatic activity to 2-KLG

wherein at least one of said first, second and third oxidative enzymatic activities requires an oxidized form of an enzymatic co-factor and said reducing enzymatic activity requires a reduced form of said enzymatic co-factor and wherein the oxidized form and the reduced form of said co-factor are recycled between at least one oxidizing step and the reducing step and said carbon source is selected from the group consisting of 6-carbon sugars, mixtures of 6-

carbon sugars, 6-carbon sugar acids, and enzymatic derivatives thereof wherein said carbon source is capable of being converted to an ASA intermediate .

Claim 19 - canceled

24.(Amended) The process of [Claim 19] Claim 18, wherein each of said first enzyme, said second enzyme and said third enzyme [is a] has dehydrogenase activity.

27.(Amended) The process of Claim 25 wherein said second enzyme [is a] has GADH activity.

28.(Amended) The process of Claim 25 wherein said third enzyme [is] has KDGDH activity.

29.(Amended) The process of Claim 25 wherein said fourth enzyme is a reductase [activity] enzyme.

36.(Amended) The process of Claim 34 wherein said host cell is non-viable.

47.(Amended) The process of Claims [1,] 15 and 18 that is continuous.

48.(Amended) The process of Claims [1,] 15 and 18 that is batch.

49.(Amended) The process of Claims [1,] 15 and 18 that proceeds in an environment comprising organic solvents.

50(Amended) The process of Claims [1,] 15 and 18 that proceeds in an environment comprising long polymers.

51. (Amended) The process of Claims [1,] 15 and 18 further comprising the step of obtaining ASA from said 2-KLG.

Claims 52 - 57 - canceled

58.(Amended) The process of [Claim 14] Claim 15 or Claim 18 wherein said 2-KLG is further purified via electrodiaysis.